PATENT ABSTRACTS OF JAPAN

(11)Publication number : 05-178756

(43)Date of publication of application : 20.07.1993

(51)Int.Cl. A61K 37/02

(21)Application number : 03-345189

(22)Date of filing : 26.12.1991

(71)Applicant : TORAY IND INC

(72)Inventor : IDA NOBUTAKA
OKAMOTO SUSUMU
OKANO KIYOSHI
SATO YUICHIRO
NARUTO MASANOBU

(54) PREVENTING AND TREATING AGENT FOR HEPATOPATHY

(57)Abstract:

PURPOSE: To obtain a preventing and therapeutic agent for hepatopathy containing interleukin 6 and/or interleukin 11 as an active ingredient.

CONSTITUTION: The objective preventing and therapeutic agent is obtained by including interleukin 6 and/or interleukin 11 as a principal ingredient. This interleukin 6 or 11 or both are obtained by culturing a cell derived from a human. This agent is administered by intervenous, subcutaneous or intramuscular injection, intravenous instillation or local infusion or transmucosal administration such as peroral, percutaneous, transpulmonary or enteral administration or the percutaneous administration. The dose is 0.0001-300μg per day and kg body weight. This agent is excellent in function to lower the glutamic-oxaloacetic transaminase(GOT) value and the glutamic-pyruvic transaminase(GPT) value raised by various hepatopathies and useful for the hepatopathies such as viral hepatitis, bacterial or parasitic infectious hepatitis, hepatopathies caused by autoimmune diseases, alcoholic hepatopathies, hepatopathies caused by medicines or poisonous substances, hepatoma or ischemic hepatopathies caused by hepatic transplantation, surgical operation or cardiac infarction.

LEGAL STATUS

[Date of request for examination] 24.12.1998

[Date of sending the examiner's decision of rejection] 03.07.2001

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to prevention and the remedy of new liver disease.
[0002]
[Description of the Prior Art] When a living body maintains HOMEO star cis- (homeostasis), the role which liver plays is very large and the object is mainly attained by metabolism. There are carbohydrate metabolism, lipid metabolism, a protein metabolic turnover, a ****** metabolic turnover, a bilirubin metabolic turnover, a vitamin metabolic turnover, a hormone metabolic turnover, drug metabolism, etc. in the metabolism of a liver, and the enzyme which participates in these processes reaches at least 1000 or more things which have become clear (a liver disease handbook, Haruo Kameda work, a MEJIKARU friend company, 1982). Thus, a liver is the core of the metabolism in a living body, and they bring [the depression and abolition are critical or] about a death-dealing result. The abnormalities in a liver function are induced by various causes. For example, the thing based on existence of the thing and neoplasm which are depended on chemicals, such as infection by the virus, bacteria, a worm, etc., a thing to depend on an autoimmune disease, an alcoholic thing, a drug, and poison, growth, etc. is various. The symptoms are similarly classified into acute hepatitis, the chronic hepatitis, fulminant hepatitis, a fatty liver, liver cirrhosis, hepatic carcinoma, etc. according to clinical findings, such as a cause and a progress situation of a condition, and a diagnosis.
[0003] As liver disease diagnostics, full use of expensive devices, such as liver scintiscanning, abdominal CT, a celiac arteriography method, a laparoscopy, and hepatobiopsy, and an advanced technique is made. However, the meaning of clinical biochemistry parameter measurement as a means to detect the abnormalities in a liver function in first is very high. There are various things in a serological liver function parameter, for example, blood serum transaminase, the blood serum alkaline phosphatase, gamma glutamyl transpeptidase, a leucine aminopeptidase, lactate dehydrogenase, choline esterase activity, etc. are the example of representation. GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) are especially contained in hepatocyte, and the transmigration is carried out into a blood flow with the denaturation of hepatocyte, and a necrosis, and since it changes sharply compared with other enzymes, it is widely used on clinical as the manifestation of liver disease, and a restorative monitor enzyme (a liver disease handbook, Haruo Kameda work, a MEJIKARU friend company, 1982).
[0004] As pharmacotherapy to liver disease, an ammonia metabolic turnover related substance and various cholekinetics, such as hormone, such as nucleic-acid precursors, such as antidotes, such as **** factors, such as an infusion solution of nutrients, such as sugar, comprehensive amino acid, and blood serum concentration albumin, a methionine, and a choline, glucuronic acid, and an SH compound, various vitamins, an orotic acid, AICA, and a nucleotide, ACTH, and adrenocorticosteroid, glutamic acid, and an aspartic acid, diuretic, etc. are prescribed for many years, for example. These answer the cause of liver disease, and a symptom, and although used together and used, independent or the results for which satisfaction dies is not necessarily acquired. In recent years, the application to the liver disease

of the peptide sex hormone called lymphokine and cytokine attracts attention. For example the effectiveness (it Immunobiolog(ies) Greenberg, H.B.et al., N.Engl.J.Med., 295, and 517- 522, 1976, Hess, and G.et al. --) of the interferon to chronic hepatitis B the possibility (Hoofnagle, J.H.et al., N.Engl.J.Med., and 315 --) of the interferon application to 172, 255-261, 1986, or C mold (un-A un-B molds) chronic hepatitis 1575-1578, 1986 Di Bisceglie, A.M.et al., N.Engl.J.Med., 321, 1506-1510, and 1986 are reported, and the expectation as a viral hepatitis remedy is growing. The proteinic discovery and the structure determination which were named HGF (Hepatocyte Growth Factor) of molecular weight 84kd which promotes the fissiparity of hepatocyte are similarly tried on in vitro (the Ichihara **, Biomedica., 6, 1151-1155, 1991), and the application as a remedy which leads liver regeneration acceleration is expected.

[0005] On the other hand, interleukin 6 (it abbreviates to IL-6 hereafter) is, Interferon beta 2 (2537 Zilberstein, A.et.at., and EMBO J. 5, 2529- 1986), B cell defferentiating factor (BSF-2): (76 T. Hirano, et.al., Nature, 324, 73- 1986), A 26-KDa protein (Hageman, G.et.al., Eur.J.Biochem., 159,625-632, 1986), A hybridoma / plasma site-Ma growth factor (919 VanDamme, J.et.al., J.Exp.Med., 165,914-1987), Hepatocyte stimulator (HSF) : (7255 221 18 -22, 1987; Andus, T.et.al., FEBS Lett., Gauldie, J.et.al., Proc.Natl.Acad.Sci.USA, 84, 7251- 1987) etc., It turned out that the physiological active substance studied independently is the same molecule, being referred to as IL-6 from the bioactive versatility was advocated, and the name is established. IL-6 promote the maturation of megakaryocyte other than the physiological function accompanying the discovery in recently and in vitro, as described above. (Ishibashi, T. et al., Proc.Natl.Acad.Sci.USA, 86, 5953-57 and 1989), and in vivo Administration reports that a platelet increases (for example, Asano, S. et al., Blood, 75, 1602-1605, 1990). [for example,]

[0006] As bioactive of IL-6, in addition, lifting of the ACTH concentration in blood (Naitoh, Y., Biochem.Biophys.Res.Commun., 155, 1459, 1988), Acceleration of the various hormone (PRL, GH, LH, FSH) production from a hypophysis (Spangeol, B., Endocrinology, 125, 575, 1989), The insulin production sthenia from the pancreas (Sandler, S., Endocrinology, 126, 1228, 1990), a nervous system -- although the acceleration of differentiation of a cell, a survival maintenance operation (Satoh, T., Mol.Cell.Biol., 8, 3546, and 1988), etc. are continuing variably The position as the matter with which these knowledge is industrially meaningful to IL-6 promptly, i.e., a remedy, is not necessarily given, and, for that purpose, rational and scientific certification is required.

[0007] About the meaning of an operation to the liver of IL-6, it is indefinite similarly. That is, IL-6 are (Andus, T., FEBS Letter, 221, 18, and 1987) as a hepatocyte stimulator, A fibrinogen, alpha1-acid glycoprotein, As a production promoter of acute stage proteins, such as alpha2-macroglobulin, (Geiger, T., Eur.J.Immunol., 18, 717, 1988, Heinrich, P., Biochem.J., 265, 621, and 1990), The report of (Ritchie, D., Am.J.Physiol., 258, E57, and 1990) as a liver glucose new promoter A certain thing, It is indefinite in whether these operations are reactions useful for a living body, or to be disadvantageous reactions, and one side. Myxoma in an atrium () [Hirano, T.,] [Proc.] In connection with Natl.Acad.Sci.USA., 84, 228-231, and 1987 and CASL Mann syndrome (Yoshizaki, K., Blood, 74, 1360-1367, and 1989), IL-6 are identified among the blood of a large quantity, The image of minus exists as a common idea. The acute stage protein production sthenia same with discovering the operation to a liver at the time of the inflammation which accompanies bacterial infection etc. to a minus image is strong.

[0008] On the other hand, interleukin 11 (it abbreviates to IL-11 hereafter) is the cytokine discovered as a blood-making factor which a stromata cell and fibroblast produce at the beginning (Paul.S.R.et al., Proc.Natl.Acad.Sci.USA, 87, 7512-7516, 1990). There is [bioactive / the] a field which is not solved in detail yet about IL-11.

[0009]

[Problem(s) to be Solved by the Invention] Originally the object of this invention is from the physiological active substance which has an operation in the living body to offer prevention and the remedy of the outstanding liver disease.

[0010]

[Means for Solving the Problem] Said object is completed by the following this inventions.

[0011] That is, this invention relates to the liver function protection which makes IL-6 and/or IL-11 an active principle, an improvement, liver disease prevention, and a liver disease therapy. This invention becomes the cause of various hepatic insufficiency and liver disease and is biological if it states in more detail. The carcinostatic substance chosen as a representative is prescribed for the patient from from among chemical and physical factors. Medicate the induced liver disease animal with IL-6 and/or IL-11, and GOT which occupies the position steadfast to detection of liver disease and a liver function abnormal condition, and a GPT value are made into an index in clinical biochemistry. IL-6 and/or liver function protection of IL-11, an improvement, liver disease prevention, and a liver disease curative effect were proved, and it was completed for the first time.
[0012] Bleedoff into the blood of GOT and GPT which are the marker enzyme of hepatocyte does not ask that it is direct and indirect, but is uniquely brought about by the denaturation of hepatocyte, and destruction. This invention showed that there was an operation which GOT which reached abnormality level in the hepatopathy model animal, and a GPT value limit to a shift or normal level in the normal level direction by administration of IL-6 and/or IL-11.
[0013] The reason using the carcinostatic substance by which current regarding as questionable is carried out as the abnormality model production approach in a liver function by this invention in the medical site is that the practical convenience of carcinostatic substance loading in completion of this invention can expect promptly according to hepatotoxicity relief of the cancer patient at the time of a carcinostatic substance activity or the object, and a symptom. Therefore, the application of this invention of be [it / what is limited only to the cancer treatment field] is clear, is especially clear also from the above-mentioned GOT and the clinical meaning of GPT enzyme activity measurement, and can be applied to the situation that all the hepatic insufficiency that may be accompanied by hepatocyte denaturation and destruction, liver disease conditions, or these stages may be arrived at, preclusively and in therapy.
[0014] There is especially no limit in IL-6 used by this invention, and IL-6 obtained by the known approach are used suitably. For example, recombinant IL-6 obtained by the thing which cultivated the IL-6 production cell and was obtained, or the modifying-gene method is sufficient. What cultivates an IL-6 production human cell and is obtained is used more preferably.
[0015] Since IL-6 which IL-6 acquired from the culture human cell can avoid mixing of the impurity of the seed origin of those other than Homo sapiens, and are obtained become a thing near IL-6 which originally work in the living body, they are desirable. That is, since an antibody production can be relatively eliminated when Homo sapiens is medicated as a remedy, since structure including a sugar chain and detailed qualification becomes a thing near IL-6 Homo sapiens in the living body, it is desirable. Therefore, IL-6 which a Homo sapiens cultured cell produces can expect in the living body and more efficient effectiveness.
[0016] If IL-6 obtained by cultivating the cell of the Homo sapiens origin are meant and being specified further, as for IL-6 which the culture human cell of this invention produces, IL-6 obtained by cultivating a normal cell, i.e., adhesion which have not cancerated (extreme transformation) or is not the cancer cell origin, human cell are desirable. IL-6 obtained according to these conditions have the structure which the sugar chain usually added. Especially although it is especially used suitably since fibroblast, the endothelial cell, the stromata cell, etc. are considered to be one of the sources of IL-6 in the living body and the part can be especially cultivated in the form near a normal cell as a normal human cell which is not the cancer cell origin, it is not limited to these.
[0017] Since especially a suitable cell is adhesion among the above-mentioned normal human cells, it can cultivate on general cell culture conditions. Although the cultivation using a common culture flask, a roller bottle, and a micro carrier (particle) etc. is used suitably, it is not limited to this. In this way, according to the usual purification method, almost pure IL-6 can be obtained from the culture medium obtained by culture of a human cell. Although an example shows the example about these culture and a purification method, of course, it is not limited to this.
[0018] On the other hand, recombinant IL-6 can be manufactured by the known approach. Although the example which made Escherichia coli the host was shown as an example as an example, it can

manufacture by using the genetic manipulation method widely learned also except this. For example, it can prepare also by connecting IL-6 gene with lower streams of rivers, such as a promotor who functions by the host, and introducing into animal cells, such as prokaryotic cells, such as a Bacillus subtilis, yeast, and a hamster cell, a mouse cell, an ape cell, a human cell, an insect cell, and an insect object with gestalten, such as DNA or a virus.

[0019] There is especially no limit in IL-11 used by this invention, and IL-11 obtained by the known approach are used suitably. For example, recombinant IL-11 obtained by the thing which cultivated the IL-11 production cell and was obtained, or the modifying-gene method is sufficient.

[0020] Recombinant IL-11 can be manufactured by the known approach. Although the example which made Escherichia coli the host was shown as an example as an example, it can manufacture by using the genetic manipulation method widely learned also except this. For example, it can prepare also by connecting IL-11 gene with lower streams of rivers, such as a promotor who functions by the host, and introducing into animal cells, such as prokaryotic cells, such as a Bacillus subtilis, yeast, and a hamster cell, a mouse cell, an ape cell, a human cell, an insect cell, and an insect object with gestalten, such as DNA or a virus.

[0021] Since IL-11 which IL-11 acquired from the culture human cell can, on the other hand, avoid mixing of the impurity of the seed origin of those other than Homo sapiens, and are obtained become a thing near IL-11 which originally work in the living body, they are desirable. That is, since an antibody production can be relatively eliminated when Homo sapiens is medicated as a remedy, since structure including a sugar chain and detailed qualification becomes a thing near IL-11 Homo sapiens in the living body, it is desirable. Therefore, IL-11 which a Homo sapiens cultured cell produces can expect in the living body and more efficient effectiveness.

[0022] If IL-11 obtained by cultivating the cell of the Homo sapiens origin are meant and being specified further, as for IL-11 which the culture human cell of this invention produces, IL-11 obtained by cultivating a normal cell, i.e., adhesion which have not cancerated (extreme transformation) or is not the cancer cell origin, human cell are desirable. IL-11 obtained according to these conditions have the structure which the sugar chain usually added. Especially although it is especially used suitably since fibroblast, the endothelial cell, the stromata cell, etc. are considered to be one of the sources of IL-11 in the living body and the part can be especially cultivated in the form near a normal cell as a normal human cell which is not the cancer cell origin, it is not limited to these.

[0023] Since especially a suitable cell is adhesion among the above-mentioned normal human cells, it can cultivate on general cell culture conditions. Although the cultivation using a common culture flask, a roller bottle, and a micro carrier (particle) etc. is used suitably, it is not limited to this. In this way, according to the usual purification method, almost pure IL-11 can be obtained from the culture medium obtained by culture of a human cell.

[0024] Prevention and the remedy of this invention contain Homo sapiens IL-6 manufactured by the approach mentioned above, and/or IL-11 as a principal component. A common excipient is chosen as other components. of course, there is no additive -- ** -- the object of this invention is attained. An additive is added for stabilization general mainly. As such an excipient, it is chosen from the protein which can be used as an excipient and/or the saccharides which were indicated by the station method. Especially suitably, out of a human serum albumin (HSA), gelatin, a mannitol, a sorbitol, a lactose, trehalose, etc., although combined and chosen, they are not proper or the thing limited to these, of course.

[0025] A living body is medicated with the constituent which uses as a principal component IL-6 obtained in this way and/or IL-11 in order to attain concretely the liver function protection which is the object of this invention, an improvement, liver disease prevention, and a liver curative effect. The hepatopathy prevention accompanying cancer chemotherapy although it does not limit especially as an object for administration, The living body which needs administration of existence of the living body which needs relief and a therapy, and the other diseases which may induce a hepatopathy directly and indirectly, or a therapy, a hepatopathy is induced -- it is -- it is -- the various kinds in which are made to carry out and it deals -- surgical or the living body which needs physical treatment -- various

hepatopathies [, such as an infectious agent, an autoimmune disease and a neoplasm,] based on a biological cause, such as a living body with the hepatopathy induction by chemicals, such as alcohol and poison, or its fear, a virus, and bacteria, were occurred -- it is -- it is -- a living body with the fear can be mentioned. As a concrete gestalt of such liver disease that can apply this invention, the ischemic hepatopathy accompanying the hepatopathy by viral hepatitis, bacteria and parasitic infectious hepatitis, the hepatopathy by the autoimmune disease, alcoholic liver injury, the drug, and poison, hepatic carcinoma or a liver transplantation, a surgical operation, myocardial infarction, etc. is mentioned. However, of course, it is not limited to the symptoms described here, and is not restrained by the existence of a complication, and the concurrence situation, either.

[0026] Although the medication method of the compound of this invention according to the symptoms here must be chosen if in charge of applying this invention to these symptoms, that does not limit the range of this invention. One [suitable] out of the general injection as a medication method, i.e., an intravenous injection, subcutaneous injection, an intramuscular injection, drop-by-drop-titration intravenous injection, partial impregnation, etc. is chosen, and it is ****. The permucosal prescribing [for the patient]-a medicine method or dermal administration methods, such as taking orally, transderma, ****, and ****, are also suitably enforced by the case.

[0027] As an effective dose, it is chosen out of per [0.0001] weight per day of 1kg in the range of 300microg. It is suitably chosen in the range of 0.001-10microg per weight of 1kg. Of course, there is no above-mentioned dose what changes also with symptoms and is limited to these values.

[0028] Although usually chosen in 1 time of the range as a count of administration on several [1 thru/or 2 times, or / 2 thru/or] for one day, it is not limited to this. When a living body is already in a hepatopathy condition as a stage prescribed for the patient so that clearly also from the result of this invention, or when there is the fear, although it is possible, it is not uniquely limited especially through the condition of the both, respectively. that is, a liver should call the organ of silence -- as for the meaning of therapy-application being used preclusively from the first, IL-6 and/or IL-11 are very larger than the data that the actual condition which cannot carry out **** detection easily, and the example of this invention showed the claustrum damage condition clearly so that it may be carried out.

[0029]

[Example] Although an example explains this invention more concretely more below at a detail, of course, this invention is not restricted by this.

[0030] In addition, the activity appraisal method of IL-6 was performed by the following approaches. The appraisal method of bioactive: Stock cell 7TD1 (IL-6 of a suitable amount are added to it using an IL-6 dependence hybridoma cell (J.vanSnick et al., European J.Immunol., 18, 193-197 (1988))) The cell proliferation of 7TD1 was measured by the MTT method, and the comparison with the growth activity about the phase dilution sample of standard IL-6 performed bioactive assessment of IL-6 separately. The same thing as being attached to the Toray Industries, Inc. Homo sapiens IL-6ELISA kit shown below as standard IL-6 was used.

[0031] The activity appraisal method of IL-11 was performed in said reference (Paul.S.R.et al., Proc.Natl.Acad.Sci.USA, 87, 7512-7516, 1990) using T1165 cell according to the approach of a publication.

[0032] ELISA (enzyme immunity appraisal method) -- law: -- anti-IL-6 antibody (N. Ida et al., Biochem.Biophys.Res.Commun., 165,728-734, (1989)) It measured by the used ELISA method. IL-6 were evaluated using the Toray Industries, Inc. manufacture, the Torre FUJIBAIONIKUSU sale, and the Homo sapiens IL-6ELISA kit.

[0033] Preparation of example 1 Escherichia-coli origin IL-6: The IL-6 expression vector which makes a frame IL-6cDNA with the same gene sequence as known reference (324 T.Hirano et al., Nature, vol. 73 (1986)) was created by the following approach.

[0034] thyroid cancer origin cell strain NIM-1 cell (a copy -- the PCR reaction was performed [mixture / which was compounded with reverse transcriptase / cDNA] by making two following DNA oligomer CCGATCGATGCCAGTACCCCCAGGA(s) and GCCACGGATCCTACATTTGCCGAAG into a primer from mRNA which cultivated **** et al., the Japanese Society of Hematology journal, 53

volumes, and 805 (1990), and was prepared by the usual approach.) It is the Escherichia coli expression vector pKM6 (Tanaka et al., J.Interferon Res., 6,429-35 (1986)) about the DNA fragment obtained after digesting the obtained magnification DNA with restriction enzymes ClaI and BamHI. It inserted between the ClaI part and the BglII part, and manifestation IL-6 vector pKMIL-6 were obtained. These pKMIL-6 were introduced into Escherichia coli HB one 101, and recombinant was obtained. This recombinant was cultivated as follows and Escherichia coli recombinant IL-6 were prepared.
[0035] Escherichia coli HB101/pKMIL-6 holding a Homo sapiens interleukin-6 manifestation plasmid were cultivated using 30L ** jar. The culture medium for growth of 30L (phosphoric-acid 1 potassium 0.3% and phosphoric-acid disodium 0.6%, 0.5% [of sodium chlorides], 0.1% [of ammonium chlorides], and glucose 0.5%, 0.5% of casamino acids, magnesium sulfate 1mM, ferrous-sulfate 3microM, vitamin B1 6microg [// ml] and ampicillin 50microg/ml) was taught to 30L ** jar, and inoculation of the above-mentioned recombinant was carried out. The jar was operated on number of stirring 300rpm, quantity-of-airflow 1VVM, and 25-degree C conditions. The Indore acrylic acid which is the inductor of a tryptophan operon was added, and it cultivated for 60 hours, adding a glucose and casamino acids. Culture biomasses were collected by centrifugal separation actuation for [10,000xg] 20 minutes. About 895g of biomasses was obtained. It is OD550nm to 50mM tris hydrochloric-acid buffer pH 8.0 which contains 1mMEDTA and 100mMNaCl for the collected biomasses. It suspended so that it might be set to 20. The biomass was crushed by MANTON gaulin, centrifugal separation was performed, and crushing extracts were collected. The amount of proteins in an extract was 235g, and interleukin -6 was 495mg. The amount of IL-6 was measured by the aforementioned ELISA method here (it is below the same).
[0036] The extract was made to stick to silica column 5.5L, and it was eluted with the acidic solution. IL-6 [462mg] were collected. The ammonium sulfate was added final concentration 1.33M to the eluate, and centrifugal removed the insoluble impurity. Next, it was made to stick to butyl column (butyl TOYOPARU TOSOH CORP. make) 200ml, and was eluted with the low-salt neutral solution. IL-6 [237mg] of 84% of purity were obtained with SDS-PAGE purity assay. IL-6 eluted were made to stick to heparin column (AF heparin TOYOPARU TOSOH CORP. make) 80ml as it is. It was eluted in the neutral salt buffer. IL-6 [114mg] of 91% of purity were obtained. The eluate was further refined again by butyl column (butyl TOYOPARU TOSOH CORP. make) 200ml, and IL-6 [66mg] were obtained. The purity of IL-6 prepared above was 95% or more by the opposite phase HPLC method. It checked that the above-mentioned IL-6 were IL-6 which had activity by the above-mentioned appraisal method.
[0037] Preparation of example 2 human-cell origin IL-6: IL-6 of this invention were adjusted by the following approach as an example. In the MEM culture medium which contains 5% of NCS of 1L in the glass cultivation tank of 2L, bead culture of the Homo sapiens fibroblast was carried out so that the number of cells might be set to 106/ml (bead: "site DEKKUSU 1", (Pharmacia Corp.), 37 degrees C). Then, the culture medium was exchanged for non-blood serum MEM culture-medium 1L containing a small amount of carboxymethyl cellulose, and the Homo sapiens natural mold interferon beta of 100,000 units / L was added as a priming. the next day -- further -- Pori l: -- poly C -- cycloheximide 10 mg/L addition was carried out 50 mg/L. 4 mg/L charge of the actinomycin-D was carried out the 4 hours after, it permuted by the MEM culture medium which contains methyl cellulose little as a production culture medium 1 more hour after, and super induction processing was performed. Culture was continued as it was for two days after that (37 degrees C).
[0038] After stopping churning and making a micro carrier sediment, the washings in supernatant liquid and a production culture medium was filtered, and 1L was moved to another container with churning equipment. "Blue Sepharose CL-6BFF" (Pharmacia Corp.) which sterilized in this production liquid is supplied, and batch adsorption was carried out, agitating for 15 degrees C and four days. After the churning halt, blue support was made to sediment and supernatant liquid was moved to another container. After carrying out autoclave sterilization (121 degrees C, 30 minutes) of the silica support in the sodium phosphate buffer solution, every 4ml two columns were filled up with it, and it was connected to the serial. To this, the bypassing supernatant liquid of blue support was passed by rate-of-flow 20 ml/hr. After carrying out a whole-quantity style, two columns were refined independently. After

pouring 25ml of sodium phosphate buffer solutions, respectively, 20mM hydrochloric acid was poured and 10ml of interleukin-6 content fractions was collected. It added so that an ammonium sulfate might be further set to 1.33M in this hydrochloric-acid recovery liquid, and 4 degrees C was agitated gently one evening. precipitate -- 3000rpm and 30-minute centrifugal separation (4 degrees C) -- it removed.
[0039] It was made to pass and stick to the column filled up with butyl TOYOPA-RU 650M"1ml (TOSOH CORP.) which is the support for hydrophobic chromatographies about the separated supernatant liquid. After 20mM hydrochloric acid which contains the ammonium sulfate of 1.33M for this column, and 50mM sodium phosphate buffer solution containing the ammonium sulfate of 1.33M washed, 50mM sodium phosphate buffer solution recovered. Then, gradient elution was carried out using the high performance chromatography (Shimazu LC-4A) equipped with the ODS column (C18) (YMC-Pack ODS A-312 S-5 120A and YMC) which is the chromatography of an opposite phase system by the water which contains triphloroacetic acid 0.1%, and the acetonitrile which contains triphloroacetic acid 0.1%, and Homo sapiens natural mold interleukin -6 peak was isolated preparatively. In this way, interleukin -6 solution which uses 5mM formic acid as a solvent, carries out the gel filtration of the obtained Homo sapiens natural mold interleukin -6 by "sephadex G-25" (Pharmacia Corp.), and does not contain an acetonitrile was obtained.
[0040] The purity of IL-6 prepared above was 95% or more by the opposite phase HPLC method. It checked that the above-mentioned IL-6 were IL-6 which had activity by the appraisal method shown above.
[0041] After medicating C57BL/6 mouse of six example 3 groups with cisplatin (it omits Following CDDP) 9.7mg/kg once intraperitoneal, 280microg/kg/day was administered hypodermically every day at 1 time per of a rate day for IL-6 from the next day. The said capacity administration of the physiological saline was carried out instead of IL-6 at the control group. It set up as one groups [six], the mouse of each group was slaughtered with time, and plasma was extracted. The result of having measured Plasma GOT was shown in a table 1.
[0042]
[A table 1]

表1 シスプラチン誘発マウス肝障害に対するIL-6の予防効果

（CDDPは実験開始0日目，IL－6は1日目から投与開始）

処置群	血漿GOT値（U／L），実験開始後；			
	実験開始前	4日目	9日目	15日目
生理食塩水群	50.7±4.9	62.2±6.2	59.0±8.9	88.2±6.2
CDDP+生理食塩水群	50.7±4.9	99.8±14.4	74.7±10.0	121.4±11.0 *
CDDP＋IL-6群	50.7±4.9	71.7±11.0	54.8±3.9	76.2±11.1

[0043] Lifting of the liver GOT value by CDDP administration is the 4th day, was already accepted, and continued till the 15th. On the other hand, by the group which used IL-6 administration of CDDP administration together, the preventive effect which a GOT value is low maintained also in which assay date, and the equivalent is mostly indicated to be the mouse group which only continued medicating cisplatin the mouse non-prescribing a medicine for the patient with a physiological saline, and shows cisplatin induction hepatotoxicity relief and liver protection was accepted clearly. Each value in a table shows the average ** standard error of n= 6, and significant difference assay is Student's. It carried out

by the T-test method. * The mark expresses p< 0.005 and ** mark expresses p< 0.01. (It is the same hereafter)

[0044] As example 4 example 3 showed, the CDDP induction hepatopathy is already discovered on the 4th day of CDDP administration. The results of a table 2 examined whether there would be any operation which reduces the GOT value which once rose to IL-6. That is, IL-6 administration initiation was performed by hypodermic administration (280microg/kg/day) every day from the 7th after CDDP administration. The plasma GOT value on the 15th (this eight-day eye) showed distinct lowering to the control group on the 9th (after IL-6 administration the 2nd day) after experiment initiation, and it was checked that there is an operation which lowers the GOT value which once rose to IL-6, i.e., a curative effect.

[0045]

[A table 2]

表2 シスプラチン誘発マウス肝障害に対するIL-6の治療効果

（CDDPは実験開始0日目，IL－6は7日目から投与開始）

処置群	血漿GOT値（U／L），実験開始後：		
	実験開始前	9日目	15日目
CDDP+生理食塩水群	50.7±4.9	79.0±15.5	89.2±12.4
CDDP＋IL-6群	50.7±4.9	45.3±4.8 *	65.5±3.6

[0046] Although example 5GOT (glutamic oxaloacetic trans aminase) is a useful blood biochemical marker which detects a hepatopathy, it is known that the value will rise also in the new disease except liver disease etc. Then, it measured by making a GPT (glutamicpyruvic transaminase) value with more high liver singularity into an index.
[0047] It is CDDP9.7mg/kg after single-dose administration and from the next day to intraperitoneal in C57BL/6 mouse (six groups) IL-6 280microg/kg/day was administered hypodermically every day and the plasma GPT value was measured with time. Four days after CDDP administration, lifting of a GPT value was observed by the result as shown in a table 3. By the group which continued at CDDP administration and prescribed IL-6 for the patient on the other hand, although there was no difference, lowering of a clear GPT value was accepted in the 15th day on the 9th in the 4th day.
[0048]
[A table 3]

表3 シスプラチン誘発マウス肝障害に対するIL-6の予防効果
（CDDPは実験開始0日目，IL－6は1日目から投与開始）

処置群	血漿GPT値（U／L），実験開始後：			
	実験開始前	4日目	9日目	15日目
生理食塩水群	19.8±1.4	28.0±2.5	20.7±1.7	29.4±3.6
CDDP+生理食塩水群	19.8±1.4	29.2±2.0	24.0±1.5	27.4±2.9
CDDP＋IL-6群	19.8±1.4	29.8±6.3	15.7±1.2 ＞**	15.4±1.5

[0049] IL-6 administration (280microg/kg/day, hypodermically, every day) was started on the 7th after example 6CDDP administration (9.7 mg/kg, intraperitoneal, single time), and the existence of a curative effect was investigated. The mouse was considered as a six group configuration by C57BL/6. As shown in a table 4, the clear GPT value reduction operation was seen by measurement on the 15th (this eight-day eye) on the 9th (after IL-6 administration the 2nd day), and the curative effect was accepted by the result.

[0050]

[A table 4]

表4 シスプラチン誘発マウス肝障害に対するIL-6の治療効果

（CDDPは実験開始0日目，IL－6は7日目から投与開始）

処置群	血漿GPT値（U／L），実験開始後：		
	実験開始前	9日目	15日目
CDDP+生理食塩水群	19.8±1.4	34.0±6.7 *	44.4±14.4
CDDP＋IL-6群	19.8±1.4	15.8±0.3	16.8±3.2

[0051] C57BL/6 mouse of six example 7 groups was medicated with mitomycin-C (it omits Following MMC) 2 mg/kg single time intraperitoneal, IL-6 of 1280microg/kg/day were administered hypodermically once per day every day from the next day, and the GPT value of the plasma which collected blood on the 7th was measured. The GPT value before experiment initiation was 24.8**1.4 units/a liter, and the GPT value on the 7th of the group which administered only the physiological saline hypodermically by MMC un-prescribing a medicine for the patient was completely equivalent to 23.0**1.5 units/the liter. On the other hand, the GPT value on the 7th of the group which used together MMC administration and physiological saline administration showed 35.3**3.5 units/the liter and the clear high price, and the hepatopathy operation of MMC appeared. However, the GPT value normalization operation with the as clear GPT value on the 7th of the group which used MMC

administration and IL-6 administration together as 21.8**3.0 units/a liter was observed.
[0052] Preparation of example 8IL-11: mRNA prepared from Homo sapiens thyroid cancer origin cell strain NIM-1 (copy **** et al., the Japanese Society of Hematology, 53 (5), 805 (1990)) From 1microg, the 2 chain cDNA was compounded for the cDNA composition kit using (BERINGA). Technique followed the approach of cDNA composition kit assignment.
[0053] Next, in order to acquire IL-11cDNA, the two DNA oligomers as follows were compounded. IL-11N CCGAATTCGGACATGAACTGTGTT IL-11C CCGAATTCGTCACAGCCGAGTCTT
[0054] PCR -- DNA thermal FUIKURA PJ1000 (PerkinElmer SHITASU) -- using -- thermal denaturation 94-degree-C for [1 minute] and annealing 50degree C -- the conditions for 2 minutes and for [chain expanding reaction 72 degrees-C] 3 minutes -- 40 cycle ******. The sample suppressed the-five effect of the variation in an experiment (reading error in PCR) using what was prepared as follows. H2 O 78.5micro liter Tenx reaction buffer 10 mu liter 10mM dNTP Mixture 2 mu liter Primer IL-11N (50 muM) 2 mu liter Primer IL-11C (50 muM) 2 mu liter cDNA 5 mu liter AmplitagTM (TAKARA SHUZO) 0.5micro liter (whole-quantity 100 mu liter)
(Tenx reaction buffer: 100mM tris and a hydrochloride (pH8.3), 500mM kCl 15mM MgCl2, 0.1% gelatin)
[0055] It chloroform[a phenol/]-processed (2 times), and chloroform processing was carried out and ethanol precipitate recovered amplified DNA. DNA is a restriction enzyme EcoRI. It cut, and low melting temperature agarose electrophoresis separated 1%, and the fragmentation of about 0.6 kbs was refined. This DNA fragment is EcoRI about vector pSRalpha (Y.Takebe et al., Mol.Cell.Biol., 8, 466-472 (1988)). It is [having ligation-carried out dephosphorization by BAP (Bacterial Alkaline Phosphatase) processing after cutting (a ligation kit activity, TAKARA SHUZO) and] Escherichia coli HB one 101. Transformation was carried out to the competent cel (TAKARA SHUZO).
[0056] DNA of five obtained clones was acquired, array analysis for a connection was performed, and IL-11 manifestation plasmid pSRIL-11 made into the object were obtained. After introducing this DNA:pSRIL -11 into COS-1 cell by the DEAE-dextran method and cultivating for two days by the culture medium containing 5% of FCS blood serums, the cell was washed by the serum free medium, the serum free medium was added, and it cultivated for 24 hours. What filtered this culture supernatant with the 0.22-micrometer filter was used for the pharmacology experiment.
[0057] C57BL/6 mouse of six example 9 groups was medicated once with CDDP907mg/kg intraperitoneal (the 0th day), and the capacity of 1100microper one per time per day 1 was administered hypodermically every day from the next day (the 1st day) for IL-11 (solution obtained in the example 8) (A group). The control group after CDDP administration was medicated with 100micro of IL-solutions 1 which carried out transfection only of the vector to the COS cell, and obtained it instead of 11 (B group). Furthermore, the group which did 100microl administration of a reference solution every day was prepared in the group which does not prescribe CDDP for the patient separately (C group). About each group, it learned from the example 3, and slaughtered and collected blood on the 9th, and Plasma GOT and GPT was measured.
[0058] Consequently, for the GOT value of 19.5**2.1 and B group, 78.1**9.5 and a GPT value were [the GOT value of A group / 57.0**7.2 and a GPT value / 54.4**6.9 and the GPT value of the GOT value of 26.3**2.8 and C group] 20.1**2.7.
[0059]
[Effect of the Invention] Since the operation which reduces the GOT value and GPT value which rose by various kinds of hepatopathies is excellent, IL-6 or IL-11 are useful as a liver function protective agent, and prevention and the remedy of liver disease.

[Translation done.]